CLAIMS

We claim:

1. A method for producing a mass-coded set of compounds of the general formula $X(Y)_n$, wherein X is a scaffold, n is from 2 to about 6, and each Y is, independently, a peripheral moiety, comprising the steps of:

(a) selecting a peripheral moiety precursor subset from a peripheral moiety precursor set, said subset comprising a sufficient number of peripheral moiety precursors that there exist at least about 250 distinct combinations of n peripheral moieties derived from said subset, wherein at least about 90% of said combinations of n peripheral moieties derived from said subset have molecular mass sums which are distinct from the molecular mass sums of all other combinations of n peripheral moieties derived from said subset; and

(b) contacting said peripheral moiety precursor subset with a scaffold precursor, said scaffold precursor having n reactive groups, wherein each reactive group is capable of reacting with at least one peripheral moiety precursor to form a covalent bond, under conditions sufficient for the reaction of each reactive group with a peripheral moiety precursor,

thereby producing a mass-coded set of compounds of the general formula $X(Y)_n$.

2. The method of Claim 1 wherein the scaffold precursor comprises one or more saturated, partially unsaturated or aromatic cyclic groups.

3. The method of Claim 2 wherein at least one cyclic group is substituted by one or more reactive groups.

4. The method of Claim 3 wherein the reactive groups are attached to the cyclic group directly or via an intervening C_{1-6}-alkylene group.

5. The method of Claim 4 wherein each reactive group is independently selected from the group consisting of: reactive carbonyl groups, reactive sulfonyl groups, reactive phosphonyl groups, terminal epoxide group and the isocyanate group.

6. The method of Claim 5 wherein the reactive group is selected from the group consisting of: carbonyl chloride, carbonyl pentafluorophenyl ester and sulfonyl chloride.

7. The method of Claim 5 wherein at least one peripheral moiety precursor comprises a primary amino group, a secondary amino group or a hydroxyl group.

8. The method of Claim 4 wherein each reactive group is independently selected from the group consisting of: primary amino, secondary amino and hydroxyl.

9. The method of Claim 8 wherein at least one peripheral moiety precursor comprises a reactive carbonyl group, reactive sulfonyl group, reactive phosphonyl group, terminal epoxide group or an isocyanate group.

10. The method of Claim 9 wherein at least one peripheral moiety precursor comprises a carbonyl chloride, a carbonyl pentafluorophenyl ester or a sulfonyl chloride group.

11. A method as claimed in Claim 1 wherein the step of selecting includes the steps of:

(a) choosing every set of two different peripheral moiety precursors from the peripheral moiety precursor set, said choosing performed in a manner such that for each set of two, if the two peripheral moiety precursors have equal molecular masses then one of the two is removed forming a remaining set;

(b) from the remaining set, choosing every set of four peripheral moiety precursors, including for a given set of four, removing one of the four peripheral moiety precursors if a sum of the molecular masses of a first two precursors in the given set of four equals a sum of the molecular masses of a second two precursors in the given set of four peripheral moiety precursors, said choosing forming a remainder set;

(c) from the remainder set, choosing every set of six different peripheral moiety precursors, including for a given set of six, removing one of the six peripheral moiety precursors if a sum of the molecular masses of a first three precursors in the given set of six equals a sum of the molecular masses of a second three precursors in the given set of six, said choosing forming a working selection set of peripheral moiety precursors from which to select a desired subset; and

(d) from the working selection set, choosing a desired subset so as to provide the selected subset by

(i) choosing a possible selected subset from the working selection set,

(ii) from the chosen possible subset, generating all possible combinations of n peripheral moiety precursors, and

(iii) determining whether the generated combinations have an acceptable percent mass redundancy, and if so, selecting the chosen possible subset as the selected subset.

12. A method as claimed in Claim 11 wherein the step of selecting is performed by a digital processor assembly.

13. A method as claimed in Claim 1 wherein the step of selecting includes selecting a peripheral moiety

precursor subset which includes peripheral moiety precursors that simultaneously produce mass-coded compounds when contacted with a scaffold precursor.

14. A method as claimed in Claim 13 wherein the step of selecting comprises the steps of:

(a) choosing every set of two different peripheral moiety precursors from the peripheral moiety precursor set, said choosing performed in a manner such that for each set of two, if the two peripheral moiety precursors have equal molecular masses then one of the two is removed forming a remaining set;

(b) from the remaining set, choosing every set of four different peripheral moiety precursors, including for a given set of four, removing one of the four peripheral moiety precursors if a sum of the molecular masses of a first two precursors in the given set of four equals a sum of the molecular masses of a second two precursors in the given set of four peripheral moiety precursors, said choosing forming a remainder set;

(c) from the remainder set, choosing every set of six different peripheral moiety precursors, including for a given set of six, removing one of the six peripheral moiety precursors if a sum of the molecular masses of a first three precursors in the given set of six equals a sum of the molecular masses of a second three precursors in the given set of six, said

choosing forming a working selection set of peripheral moiety precursors from which to select a desired subset; and

(d) from the working selection set, choosing a desired subset so as to provide the selected subset by

(i) choosing a possible selected subset from the working selection set,

(ii) from the chosen possible subset, generating all possible combinations of n peripheral moiety precursors, and

(iii) determining whether the generated combinations have an acceptable percent mass redundancy, and if so, selecting the chosen possible subset as the selected subset.

15. A method as claimed in Claim 14 wherein the step of selecting is performed by a digital processor assembly.

16. A method for identifying a member of a mass-coded combinatorial library which is a ligand for a biomolecule, said mass-coded molecular library comprising compounds of the general formula XY_n, wherein n is an integer from 2 to about 6, X is a scaffold and each Y is, independently, a peripheral moiety, wherein said mass-coded combinatorial library is produced by reacting a scaffold precursor with a sufficient number of distinct peripheral moiety precursors such that there exist

at least about 250 distinct combinations of n peripheral moieties derived from said peripheral moiety precursors, said method comprising the steps of:

(a) contacting the biomolecule with the mass-coded molecular library, whereby members of the mass-coded molecular library which are ligands for the biomolecule bind to the biomolecule to form biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the biomolecule remain unbound;

(b) separating the biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(c) dissociating the biomolecule-ligand complexes; and

(d) determining the molecular mass of each ligand to identify the set of n peripheral moieties present in each ligand,

wherein the molecular mass of each ligand corresponds to a set of n peripheral moieties present in that ligand, thereby identifying a member of the mass-coded combinatorial library which is a ligand for the biomolecule.

17. The method of Claim 16 wherein the biomolecule is immobilized on a solid support.

18. The method of Claim 17 wherein the solid support is a water-insoluble matrix contained within a chromatographic column.

19. The method of Claim 16 wherein a solution comprising the biomolecule is contacted with the mass-coded molecular library to form, if one or more members of the mass-coded ~~molecular~~ combinatorial library are ligands for the biomolecule, a solution comprising biomolecule-ligand complexes and unbound members of the mass-coded ~~molecular~~ combinatorial library.

20. The method of Claim 19 wherein the unbound members of the mass-coded ~~molecular~~ combinatorial library are separated from the biomolecule-ligand complexes by directing the solution comprising biomolecule-ligand complexes and the unbound members of the mass-coded ~~molecular~~ combinatorial library through a size exclusion chromatography column, whereby the unbound members of the mass-coded ~~molecular~~ combinatorial library elute from said column after the biomolecule-ligand complexes.

21. The method of Claim 19 wherein the unbound members of the mass-coded ~~molecular~~ combinatorial library are separated from the biomolecule-ligand complexes by contacting the solution comprising biomolecule-ligand complexes and the unbound members of the mass-coded ~~molecular~~ combinatorial library with a size-exclusion membrane, whereby the unbound ~~compounds~~ compounds pass through said membrane and the biomolecule-ligand complexes do not pass through said membrane.

22. The method of Claim 16 wherein the biomolecule is a protein or a nucleic acid molecule.

23. A method for identifying a member of a mass-coded combinatorial library which is a ligand for a biomolecule and assessing the the effect of the binding of the ligand to the biomolecule, said mass-coded molecular library comprising compounds of the general formula XY_n, wherein n is an integer from 2 to about 6, X is a scaffold and each Y is, independently, a peripheral moiety, wherein said mass-coded combinatorial library is produced by reacting a scaffold precursor with a sufficient number of distinct peripheral moiety precursors such that there exist at least about 250 distinct combinations of n peripheral moieties derived from said peripheral moiety precursors, said method comprising the steps of:

(a) contacting the biomolecule with the mass-coded molecular library, whereby members of the mass-coded molecular library which are ligands for the biomolecule bind to the biomolecule to form biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the biomolecule remain unbound;

(b) separating the biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(c) dissociating the biomolecule-ligand complexes;

(d) determining the molecular mass of each ligand to identify the set of n peripheral moieties present in each ligand,

wherein the molecular mass of each ligand corresponds to a set of n peripheral moieties

present in that ligand, thereby identifying a member of the mass-coded combinatorial library which is a ligand for the biomolecule; and

(e) assessing in an *in vitro* assay the effect of the binding of the ligand to the biomolecule on the function of the biomolecule.

24. The method of Claim 23 wherein the *in vitro* assay is a cell proliferation assay, a cell death assay or a viral replication assay.

25. The method of Claim 23 wherein the biomolecule is a protein or a nucleic acid molecule.

26. A method for identifying a member of a mass-coded combinatorial library which is a ligand for a biomolecule and assessing the the effect of the binding of the ligand to the biomolecule, said mass-coded molecular library comprising compounds of the general formula XY_n, wherein n is an integer from 2 to about 6, X is a scaffold and each Y is, independently, a peripheral moiety, wherein said mass-coded combinatorial library is produced by reacting a scaffold precursor with a sufficient number of distinct peripheral moiety precursors such that there exist at least about 250 distinct combinations of n peripheral moieties derived from said peripheral moiety precursors, said method comprising the steps of:

(a) contacting the biomolecule with the mass-coded molecular library, whereby members of the

mass-coded molecular library which are ligands for the biomolecule bind to the biomolecule to form biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the biomolecule remain unbound;

(b) separating the biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(c) dissociating the biomolecule-ligand complexes;

(d) determining the molecular mass of each ligand to identify the set of n peripheral moieties present in each ligand,

wherein the molecular mass of each ligand corresponds to a set of n peripheral moieties present in that ligand, thereby identifying a member of the mass-coded combinatorial library which is a ligand for the biomolecule; and

(e) assessing in an *in vivo* assay the effect of the binding of the ligand to the biomolecule on the function of the biomolecule.

27. The method of Claim 26 wherein the effect of the binding of the ligand to the biomolecule on the function of the biomolecule is assessed in an animal model, in an organism or in a human.

28. The method of Claim 26 wherein the biomolecule is a protein or a nucleic acid molecule.

29. A method for identifying a member of a mass-coded molecular library which is a ligand for a

biomolecule and bind to the biomolecule at the binding site of a known second ligand for the biomolecule, said mass-coded molecular library comprising compounds of the general formula XY_n, wherein n is an integer from 2 to about 6, X is a scaffold and each Y is, independently, a peripheral moiety, wherein said mass-coded molecular library is produced by reacting a scaffold precursor with a sufficient number of distinct peripheral moiety precursors such that there exist at least about 250 distinct combinations of n peripheral moieties derived from said peripheral moiety precursors, said method comprising the steps of:

(a) contacting the biomolecule with the mass-coded molecular library, whereby members of the mass-coded molecular library which are ligands for the biomolecule bind to the biomolecule to form biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the biomolecule remain unbound;

(b) separating the biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(c) contacting the biomolecule-ligand complexes with the second ligand to dissociate biomolecule-ligand complexes in which the ligand binds to the biomolecule at the binding site of the second ligand, thereby forming biomolecule-second ligand complexes and dissociated ligands;

(d) separating the dissociated ligands and biomolecule-ligand complexes; and

(e) determining the molecular mass of each dissociated ligand,

wherein the molecular mass of each dissociated ligand corresponds to a set of peripheral moieties present in that ligand, thereby identifying a member of the mass-coded molecular library which is a ligand for the biomolecule and binds to the biomolecule at the binding site of the known second ligand for the biomolecule.

30. The method of Claim 29 wherein the second ligand is a polypeptide, a nucleic acid molecule or a cofactor.

31. The method of Claim 29 wherein wherein the biomolecule is immobilized on a solid support.

32. The method of Claim 31 wherein the solid support is a water-insoluble matrix contained within a chromatographic column.

33. The method of Claim 29 wherein the biomolecule is a protein or a nucleic acid molecule.

34. A method for identifying a member of a mass-coded combinatorial library which is a ligand for a first biomolecule but is not a ligand for a second biomolecule, said mass-coded molecular library comprising compounds of the general formula XY_n,

wherein n is an integer from 2 to about 6, X is a scaffold and each Y is, independently, a peripheral moiety, wherein said mass-coded combinatorial library is produced by reacting a scaffold precursor with a sufficient number of distinct peripheral moiety precursors such that there exist at least about 250 distinct combinations of n peripheral moieties derived from said peripheral moiety precursors, said method comprising the steps of:

(a) contacting the first biomolecule with the mass-coded molecular library, whereby members of the mass-coded molecular library which are ligands for the first biomolecule bind to the first biomolecule to form first biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the first biomolecule remain unbound;

(b) separating the first biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(c) dissociating the first biomolecule-ligand complexes;

(d) determining the molecular mass of each ligand for the first biomolecule;

(e) contacting the second biomolecule with the mass-coded molecular library, whereby members of the mass-coded molecular library which are ligands for the second biomolecule bind to the second biomolecule to form second biomolecule-ligand complexes and members of the mass-coded

library which are not ligands for the second biomolecule remain unbound;

(f) separating the second biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(g) dissociating the second biomolecule-ligand complexes;

(h) determining the molecular mass of each ligand for the second biomolecule; and

(i) determining which molecular mass or masses determined in step (d) are not determined in step (h), thereby providing the molecular masses of members of the mass-coded combinatorial library which are ligands for the first biomolecule but are not ligands for the second biomolecule,

wherein the each molecular mass determined in step (i) corresponds to a set of n peripheral moieties present in a ligand for the first biomolecule which is not a ligand for the second biomolecule, thereby identifying a member of the mass-coded combinatorial library which are ligands for the first biomolecule but are not ligands for the second biomolecule.

35. The method of Claim 34 wherein the first and second biomolecules are each, independently, a protein or a nucleic acid molecule.

36. The method of Claim 35 wherein the first and second biomolecules are each a protein and amino acid

sequence of the second biomolecule is derived from the amino acid sequence of the first biomolecule by insertion, deletion or substitution of one or more amino acid residues.

37. The method of Claim 35 wherein the first biomolecule is a first protein and the second biomolecule is a second protein, said first and second proteins having the same amino acid sequence, wherein said first and second proteins have different posttranslational modifications.

38. The method of Claim 37 wherein the first protein differs from the second protein in extent of phosphorylation, glycosylation or ubiquitination.

39. The method of Claim 35 wherein the second biomolecule is a complex of the first biomolecule with a ligand.

40. The method of Claim 35 wherein the first and second biomolecules are each immobilized on a solid support.

41. The method of Claim 40 wherein the solid support is a water-insoluble matrix contained within a chromatographic column.

42. The method of Claim 35 wherein a solution comprising the first biomolecule is contacted with the mass-coded molecular library to form a solution

comprising first biomolecule-ligand complexes and unbound members of the mass-coded molecular library and a solution comprising the second biomolecule is contacted with the mass-coded molecular library to form a solution comprising second biomolecule-ligand complexes and unbound members of the mass-coded molecular library.

43. The method of Claim 42 wherein the unbound members of the mass-coded molecular library are separated from the second biomolecule-ligand complexes by directing the solution comprising second biomolecule-ligand complexes and the unbound members of the mass-coded molecular library through a size exclusion chromatography column, whereby the unbound members of the mass-coded molecular library elute from said column after the second biomolecule-ligand complexes.

44. The method of Claim 42 wherein the unbound members of the mass-coded molecular library are separated from the second biomolecule-ligand complexes by contacting the solution comprising second biomolecule-ligand complexes and the unbound members of the mass-coded molecular library with a size-exclusion membrane, whereby the unbound combounds pass through said membrane and the second biomolecule-ligand complexes do not pass through said membrane.

45. A method for identifying a member of a mass-coded combinatorial library which is a ligand for a first biomolecule but is not a ligand for a second biomolecule, said mass-coded molecular library comprising compounds of the general formula XY_n, wherein n is an integer from 2 to about 6, X is a scaffold and each Y is, independently, a peripheral moiety, wherein said mass-coded combinatorial library is produced by reacting a scaffold precursor with a sufficient number of distinct peripheral moiety precursors such that there exist at least about 250 distinct combinations of n peripheral moieties derived from said peripheral moiety precursors, said method comprising the steps of:

(a) contacting the second biomolecule with the mass-coded molecular library, whereby members of the mass-coded molecular library which are ligands for the second biomolecule bind to the second biomolecule to form second biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the second biomolecule remain unbound;

(b) separating the second biomolecule-ligand complexes from the unbound members of the mass-coded molecular library;

(c) contacting the first biomolecule with the unbound members of the mass-coded molecular library of step (b), whereby members of the mass-coded molecular library which are ligands for the first biomolecule bind to the first

biomolecule to form first biomolecule-ligand complexes and members of the mass-coded library which are not ligands for the first biomolecule remain unbound;

(d) dissociating the first biomolecule-ligand complexes;

(e) determining the molecular mass of each ligand for the first biomolecule;

wherein each molecular mass determined in step (e) corresponds to a set of n peripheral moieties present in a ligand for the first biomolecule which is not a ligand for the second biomolecule, thereby identifying a member of the mass-coded combinatorial library which is a ligand for the first biomolecule but are is not a ligand for the second biomolecule.

46. The method of Claim 45 wherein the first and second biomolecules are each, independently, a protein or a nucleic acid molecule.

47. The method of Claim 45 wherein the second biomolecule is immobilized on a solid support.

48. The method of Claim 47 wherein the solid support is a water-insoluble matrix contained within a chromatographic column.

49. Apparatus for producing a mass-coded set of compounds of the general formula $X(Y)_n$, wherein X

is a scaffold, n is from 2 to about 6, and each Y is, independently, a peripheral moiety, comprising a digital processor assembly for selecting a peripheral moiety precursor subset from a peripheral moiety precursor set, said subset comprising a sufficient number of peripheral moiety precursors that there exist at least about 250 distinct combinations of n peripheral moieties derived from said subset, wherein at least about 90% of said combinations of n peripheral moieties derived from said subset have molecular mass sums which are distinct from the molecular mass sums of all other combinations of n peripheral moieties derived from said subset.

50. Apparatus as claimed in Claim 49 wherein the digital processor assembly employs a routine executed by a digital processor to:

(a) choose every set of two different peripheral moiety precursors from the peripheral moiety precursor set, said choosing performed in a manner such that for each set of two, if the two peripheral moiety precursors have equal molecular masses then one of the two is removed forming a remaining set;

(b) from the remaining set, choose every set of four peripheral moiety precursors, including for a given set of four, removing one of the four peripheral moiety precursors if a sum of the molecular masses of a first two precursors

in the given set of four equals a sum of the molecular masses of a second two precursors in the given set of four peripheral moiety precursors, said choosing forming a remainder set;

(c) from the remainder set, choose every set of six different peripheral moiety precursors, including for a given set of six, removing one of the six peripheral moiety precursors if a sum of the molecular masses of a first three precursors in the given set of six equals a sum of the molecular masses of a second three precursors in the given set of six, said choosing forming a working selection set of peripheral moiety precursors from which to select a desired subset; and

(d) from the working selection set, choose a desired subset so as to provide the selected subset by

(i) choosing a possible selected subset from the working selection set,

(ii) from the chosen possible subset, generating all possible combinations of n peripheral moiety precursors, and

(iii) determining whether the generated combinations have an acceptable percent mass redundancy, and if so, selecting the chosen possible subset as the selected subset.

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